

### **Amendments to the Claims**

This listing of claims will replace all prior versions, and listings, of claims in the application:

#### **Listing of Claims:**

Claim 1 (currently amended): A method for separating a nucleic acid containing both a first nucleotide sequence type and a second nucleotide sequence type from a sample also comprising nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type, said method comprising:

(a) coupling a first hybridization probe configured for hybridizing to the first nucleotide sequence type to a magnetically responsive first bead via a first pair of complexing agents to form a first probe-bead complex, and coupling a second hybridization probe configured for hybridizing to the second nucleotide sequence type to a magnetically non-responsive second bead, which is distinguishable from the first bead by size, charge, color, or attachability to a solid support, via a second pair of complexing agents to form a second probe-bead complex;

(b) mixing the first probe-bead complex and the second probe-bead complex with the sample to form a mixture under conditions such that the first hybridization probe hybridizes to the first nucleotide sequence type and the second hybridization probe hybridizes to the second nucleotide sequence type;

(c) separating the first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof from the first mixture by applying magnetic force to the mixture and then washing the isolated first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof, thereby obtaining a fraction comprising the first nucleotide sequence type;

(d) separating the second probe-bead complex and nucleic acids hybridized to the second hybridization probe portion thereof from the fraction comprising the first nucleotide sequence type according to the properties of the distinguishable feature of the second bead and then washing to remove nucleic acids not hybridized to the second hybridization probe, thereby separating the nucleic acid containing both the first nucleotide sequence type and the second

nucleotide sequence type from nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type.

Claim 2 (original): A method for separating and quantifying a nucleic acid containing both a first nucleotide sequence type and a second nucleotide sequence type from a sample also comprising nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type comprising:

(a) coupling a first hybridization probe configured for hybridizing to the first nucleotide sequence type to a magnetically responsive first bead via a first pair of complexing agents to form a first probe-bead complex, and coupling a second hybridization probe configured for hybridizing to the second nucleotide sequence type to a magnetically non-responsive second bead, which comprises a feature distinguishable from the first bead by size, charge, color, or attachability to a solid support, via a second pair of complexing agents to form a second probe-bead complex;

(b) mixing the first probe-bead complex and the second probe-bead complex with the sample to form a mixture under conditions such that the first hybridization probe hybridizes to the first nucleotide sequence type and the second hybridization probe hybridizes to the second nucleotide sequence type;

(c) separating the first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof from the mixture by applying magnetic force to the mixture for isolating the first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof and then washing the isolated first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof, thereby obtaining a fraction comprising the first nucleotide sequence type;

(d) separating the second probe-bead complex and nucleic acids hybridized to the second hybridization probe portion thereof from the fraction comprising the first nucleotide sequence type according to the properties of the distinguishable feature of the second bead and then washing to remove nucleic acids not hybridized to the second hybridization probe, thereby separating the nucleic acid containing both the first nucleotide sequence type and the second

nucleotide sequence type from nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type; and

(e) determining the amount of nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type as a proportion of total nucleic acids present in the sample.

Claim 3 (currently amended): A method for diagnosing a disease or disorder associated with the presence in an individual of a nucleic acid comprising a first nucleotide sequence type and a second nucleotide sequence type comprising:

(a) coupling a first hybridization probe configured for hybridizing to the first nucleotide sequence type to a magnetically responsive first bead via a first pair of complexing agents to form a first probe-bead complex, and coupling a second hybridization probe configured for hybridizing to the second nucleotide sequence type to a magnetically non-responsive second bead via a second pair of complexing agents to form a second probe-bead complex;

(b) obtaining a nucleic acid sample from an individual to be tested and mixing the first probe-bead complex and the second probe-bead complex with the sample to form a mixture under conditions such that the first hybridization probe hybridizes to the first nucleotide sequence type and the second hybridization probe hybridizes to the second nucleotide sequence type;

(c) separating the first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof from the first mixture by applying magnetic force to the first mixture for isolating the first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof and then washing the isolated first probe-bead complex and nucleic acids hybridized to the first hybridization probe portion thereof, thereby obtaining a fraction comprising the first nucleotide sequence type; and

(d) separating the second probe-bead complex and nucleic acids hybridized to the second hybridization probe portion thereof from the fraction comprising the first nucleotide sequence type according to the properties of the distinguishable feature of the second bead and then washing to remove nucleic acids not hybridized to the second hybridization probe, thereby

separating the nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type from nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type; ~~and~~

(e) detecting the presence of the nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type; and

(f) quantifying the nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type, wherein the presence or increased presence of the nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type supports a diagnosis of the presence of the disease or disorder, and wherein the absence or decreased presence of a nucleic acid containing both the first nucleotide sequence type and the second nucleotide sequence type supports a diagnosis of the absence of the disease or disorder.

Claim 4 (currently amended): A kit for separating a nucleic acid containing both a first nucleotide sequence type and a second nucleotide sequence type from a sample comprising nucleic acids not containing both the first nucleotide sequence type and the second nucleotide sequence type comprising:

(a) a first probe-bead complex comprising a first hybridization probe configured for hybridizing to the first nucleotide sequence type coupled to a magnetically responsive first bead via a first pair of complexing agents, ~~and~~

(b) a second probe-bead complex comprising a second hybridization probe configured for hybridizing to the second nucleotide sequence type coupled to a magnetically non-responsive second bead, which comprises a feature distinguishable from the first bead by size, charge, color, or attachability to a solid support, via a second pair of complexing agents[.]; and

(c) instructions for using the first probe-bead complex and the second probe-bead complex to separate a nucleic acid containing a first nucleotide sequence type and a second nucleotide sequence type from a sample comprising the steps of mixing the first and second probe-bead complexes with the sample to form a mixture, separating the first probe-bead complex and nucleic acids hybridized thereto from the mixture using a magnetic force to obtain a

first nucleotide sequence fraction, and separating the second probe-bead complex and nucleic acids hybridized thereto from the first nucleotide sequence fraction according to the properties of the distinguishable feature of the second bead to obtain a nucleotide fraction of sequences containing nucleic acids containing both the first and second nucleotide sequence types.

Claim 5 (original): A process for separating objects bearing at least a first binding site and a second binding site from other objects that do not bear both said first binding site and said second binding site, comprising

(a) mixing a first binder/bead composition, comprising a magnetically responsive bead coupled to a first binder that binds the first binding site on said objects, and a second binder/bead composition, comprising a magnetically non-responsive bead coupled to a second binder that binds the second binding site on said objects, wherein said magnetically non-responsive bead comprises a feature distinguishable from the first bead by size, charge, color, or attachability to a solid support, with a fluid containing said objects and said other objects to form a mixture such that said first binder binds said first binding site to form a first complex and said second binder binds said second binding site to form a second complex;

(b) contacting said mixture with a magnetic field such that said first complex is attracted to said magnetic field and removing said first complex from said mixture to form a fraction; and

(c) separating said objects from said fraction comprising said other objects by the size, charge, color, or attachability to a solid support that distinguishes the magnetically non-responsive bead from the magnetically responsive bead.

Claim 6 (original): The process of claim 5 wherein said objects are selected from the group consisting of nucleic acids, proteins, chromosomes, cells, and organelles.

Claim 7 (withdrawn): A method of quantifying a desired protein in a sample comprising the steps of:

- (a) coating a solid support with a first antibody that recognizes the desired protein to create an antibody-coated solid support;
  - (b) contacting the antibody-coated solid support with the sample to allow the first antibody to bind to the desired protein;
  - (c) attaching a second antibody to the desired protein, wherein the second antibody comprises a modification such that the second antibody may be attached to a first set of microbeads;
  - (d) attaching first set of microbeads to the second antibody;
  - (e) washing to remove microbeads that are not attached to the second antibody; and
  - (f) counting the microbeads that are attached to the second antibody to determine the quantity of the desired protein;
- and wherein steps (a) – (d) may be performed in any order or simultaneously.

Claim 8 (withdrawn): The method of claim 7, wherein the solid support comprises a second set of microbeads that is distinguishable from the first set of microbeads by size, charge, color, or attachability to a solid support.

Claim 9 (withdrawn): The method of claim 8, wherein the second set of microbeads is distinguishable from the first set of microbeads by size.

Claim 10 (withdrawn): The method of claim 7, wherein the modification comprises a biotin molecule and the first set of microbeads comprise streptavidin.

Claim 11 (withdrawn): The method of claim 7, further comprising the step of releasing the microbeads attached to the second antibody by protease treatment prior to the counting step.

Claim 12 (withdrawn): A method of quantifying a desired protein in a sample comprising the steps of:

- (a) coating a solid support with a first antibody that recognizes the desired protein to create an antibody-coated solid support;
- (b) contacting the antibody-coated solid support with the sample to allow the first antibody to bind to the desired protein;
- (c) attaching a second antibody to the desired protein, wherein the second antibody is attached to a microbead;
- (d) washing to remove second antibody that is not attached to the desired protein; and
- (e) counting the microbeads to determine the quantity of the desired protein.

Claim 13 (withdrawn): The method of claim 12, further comprising the step of releasing the microbeads attached to the second antibody by protease treatment prior to the counting step.

Claim 14 (withdrawn): A method for quantifying a target nucleic acid molecule in a sample, wherein the target nucleic acid molecule comprises a first sequence unique to a first region of the target nucleic acid molecule and a second sequence unique to a second region of the target nucleic acid molecule, said method comprising the steps of:

- (a) contacting a first hybridization probe, which comprises a nucleic acid sequence complementary to the first region, with the sample of nucleic acids under conditions favorable for hybridization such that the first hybridization probe hybridizes to the first sequence;
- (b) contacting a second hybridization probe, which comprises a nucleic acid sequence complementary to the second region, with the sample of nucleic acids under conditions favorable for hybridization such that the second hybridization probe hybridizes to the second sequence;
- (c) immobilizing the first hybridization probe to a solid support by forming a linkage between the first hybridization probe and the solid support;
- (d) washing to remove unattached first and second hybridization probes and unattached target nucleic acids;
- (e) attaching a microbead to the second hybridization probe by forming a linkage between the second hybridization probe and the microbead;
- (f) washing to remove unattached microbeads;

(g) digesting the nucleic acid molecule with DNase or RNase to free the microbeads;  
and

(h) counting the microbeads to determine the quantity of the target nucleic acid molecule;

and wherein steps (a) and (b) may be performed simultaneously in the same hybridization solution.

Claim 15 (withdrawn): The method of claim 14, wherein the microbeads are counted using a particle counting system.

Claim 16 (withdrawn): The method of claim 15, wherein the particle counting system comprises a Multisizer II by Coulter, Inc.

Claim 17 (withdrawn): The method of claim 14, wherein the microbeads comprise fluorescent tags and wherein the microbeads are counted using a fluorescence detection system.

Claim 18 (withdrawn): The method of claim 17, wherein the microbeads are counted using a flow cytometer by measuring fluorescence emitted from beads.

Claim 19 (withdrawn): The method of claim 14, wherein the microbeads are counted by chemiluminescence or electrochemiluminescence methods.

Claim 20 (withdrawn): A method for quantifying target nucleic acid molecules in a sample, wherein said target nucleic acid molecules comprise a first nucleotide region and a second nucleotide region that comprises one or more target nucleotide sequences up to a number, N, of target nucleotide sequences, said method comprising the steps of:

(a) adding a probe mixture comprising N types of hybridization probes to the sample to create a hybridization mixture, wherein each hybridization probe comprises a first nucleotide sequence complementary to the first nucleotide region of the target nucleic acid molecules and a



second nucleotide sequence that is complementary to one of the target nucleotide sequences, and wherein each hybridization probe is coupled to a first probe-bound complexing agent and a second probe-bound complexing agent, such that all hybridization probes comprising a particular target nucleotide sequence are coupled to identical second complexing agents;

(b) adding a microparticle mixture comprising N types of microparticles to the hybridization mixture, wherein each type of microparticle comprises a microparticle-bound complexing agent specific for a particular second probe-bound complexing agent and wherein different types of microparticles are of different sizes, such that microparticles comprising microparticle-bound complexing agents specific for a particular second probe-bound complexing agent attach to nucleic acid molecules comprising the particular target nucleotide sequence;

(c) separating nucleic acid molecules that comprise a first nucleotide region from the mixture by complexing the nucleic acid molecules that comprise the first nucleotide region on a solid support, wherein the solid support comprises a support-bound complexing agent that binds to the first probe-bound complexing agent, such that microparticles attached to a nucleic acid molecule comprising both the first nucleotide region and the particular target nucleotide sequence are complexed to the solid support;

(d) washing to remove unattached microparticles;

(e) freeing microparticles that are complexed to the solid support; and

(f) counting the number of each type of microparticle to determine the number of each type of target nucleotide sequence in the sample.

Claim 21 (withdrawn): The method of claim 20, wherein the second probe-bound complexing agents comprise peptides and the microparticle-bound complexing agents comprise antibodies.

Claim 22 (withdrawn): The method of claim 21, wherein the antibodies are biotinylated.

Claim 23 (withdrawn): The method of claim 20, wherein the microparticles are polystyrene beads.

Claim 24 (withdrawn): The method of claim 23, wherein the microparticles range from about 1 to about 20  $\mu\text{m}$  in diameter.

Claim 25 (withdrawn): The method of claim 20, wherein the step of freeing the microparticles comprises adding a protease to digest the microparticle-bound complexing agents.

Claim 26 (original): The method of claim 1, wherein the solid support comprises a magnetically-responsive microbead.